Sentenced to senescence - bringing rebellious cells to justice

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CHAPTER 1

GENERAL INTRODUCTION

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CELLULAR METABOLIC PATHWAYS

Homeostasis of energy is the basis of all life: cells and organisms survive only because of the ability to give-and-take energy. Adenosine triphosphate (ATP) transports chemical energy within cells and is therefore an essential cellular currency providing energy for countless energy-consuming processes such as (macro) molecular biosynthesis, transport and motility. The main source of cellular ATP is the oxidation of carbons such as glucose and lipids[1].

Glucose enters the cell via glucose transporters (GLUTs) and is subsequently phosphorylated to glucose-6-phosphate (G6P) by hexokinases (HKs), which prevents its exit from the cell[1]. G6P can either proceed to glycolysis, producing ATP, NADH and pyruvate, or it can enter the pentose phosphate pathway (PPP, Figure 1). In addition to hexokinases, enzymes essential for the stimulation and suppression of glycolysis are 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFBs), phosphofructokinases (PFKs), phosphoglycerate mutase (PGM) and pyruvate kinases (PKs). Under anaerobic conditions, glucose-derived pyruvate is converted by lactate dehydrogenase (LDH) to lactic acid in a process called anaerobic glycolysis. In the presence of oxygen, the activity of latter enzyme is usually decreased and pyruvate is metabolized in the mitochondria to acetyl-CoA by pyruvate dehydrogenase (PDH), thereby fueling the tricarboxylic acid (TCA) cycle (Figure 1). Oxidation of acetyl-CoA in the TCA cycle converts NAD^+ into NADH and FAD into $FADH_2$, which subsequently generate ATP in a process called oxidative phosphorylation (OxPhos)[2]. Reactive oxygen species (ROS), by-products of OxPhos, can act as essential signaling molecules, but when present at high levels they can also cause irreversible oxidation of DNA, proteins and lipids[1].

Several glycolytic and TCA intermediates may be used for biosynthesis of macromolecules required for the cell cycle progression such as amino acids, lipids and nucleotides[3]. For fatty acid (FA) synthesis, the TCA intermediate citrate can be transported to the cytosol and converted into cytosolic acetyl-CoA by an enzyme called ATP-citrate lyase (ACLY)[1,4]. By the activity of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) enzymes, cytosolic acetyl-CoA can be metabolized into the FAs (Figure 1). In addition, the glycolysis intermediate dihydroxyacetone phosphate (DHAP) can fuel lipogenesis, the main method for storing glucose in adipocytes[1,4].

A major source of nucleotides is the PPP. It shunts G6P away from glycolysis by converting it into ribose-5-phosphate (R5P) in a reaction carried out by the rate-limiting G6P dehydrogenase (G6PDH) enzyme (Figure 1)[5]. By doing so, the PPP does not only provide nucleotide precursors, but is also the primary supplier of NADPH. NADPH is a cofactor required for reductive biosynthesis of FAs, amino acids and nucleotides, but also regulates the cellular redox status by reducing the antioxidant glutathione.

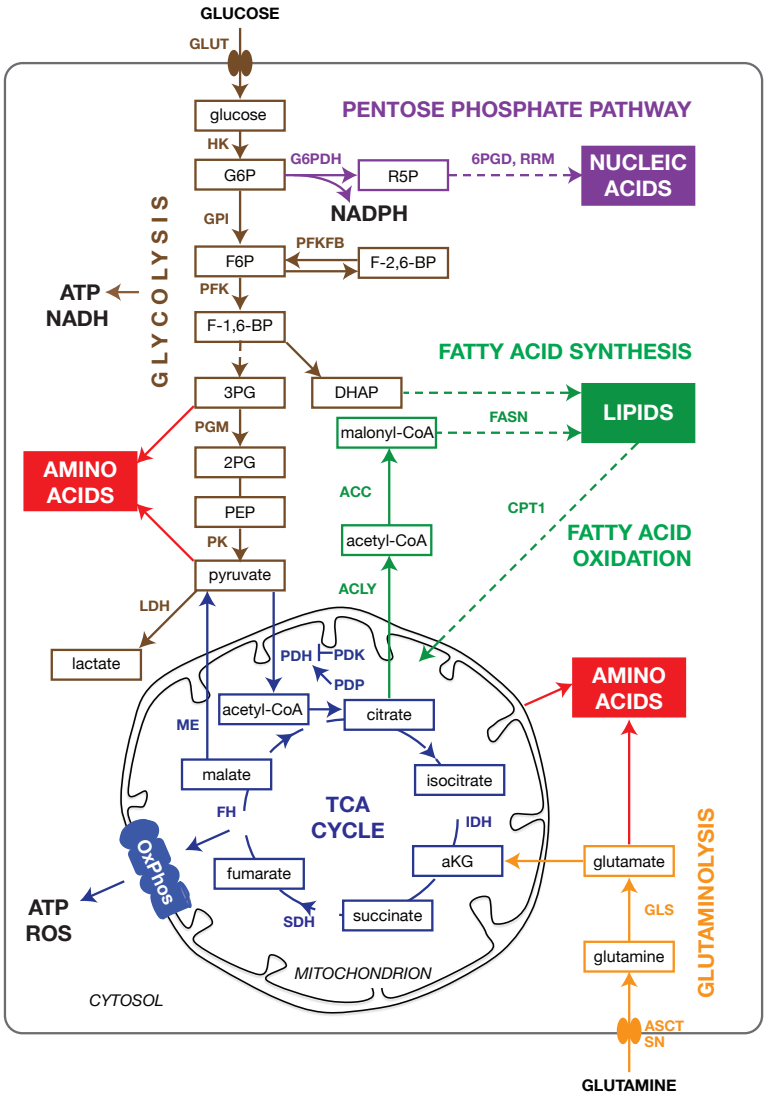

Figure 1. Schematic representation of major metabolic pathways

2PG - 3-phosphoglycerate; 3PG - 3-phosphoglycerate; 6PGD - 6-phosphogluconate dehydrogenase; ACC - acetyl-CoA carboxylase; ACLY - ATP-citrate lyase; ASCT - Alanine/Serine/Cysteine/Threonine amino-acid transporter; ATP - adenosine triphosphate; CPT1 - carnitine palmitoyltransferase 1; DHAP - dihydroxyacetone phosphate; F-1,6-BP - fructose-1,6-bisphosphate; F-2,6-BP, fructose-2,6-bisphosphate; F6P - fructose-6-phosphate; FASN - fatty acid synthase; FH - fumarate hydratase; G6P - glucose-6-phosphate; G6PDH - glucose-6-phosphate dehydrogenase; GLS - glutaminase; GLUT - glucose transporter; GPI - glucosephosphate isomerase; HK - hexokinase; IDH - isocitrate dehydrogenase; αKG - α-ketoglutarate; LDH - lactate dehydrogenase; ME - malic enzyme; PDK - pyruvate dehydrogenase kinase; PDH - pyruvate dehydrogenase; PDP - pyruvate dehydrogenase phosphatase; PEP - phosphoenolpyruvate; PFK - phosphofructokinase; PFKFB - 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; PGM - phosphoglycerate mutase; PK - pyruvate kinase; R5P - ribose-5-phosphate; RRM- ribonucleotide reductase M; ROS - reactive oxygen species; SDH- succinate dehydrogenase; SN - system N transporter; TCA cycle - tricarboxylic acid cycle.

Amino acids can be derived from the TCA intermediates citrate, α-ketoglutarate (αKG) and malate, as well as the glycolytic intermediate pyruvate and 3-phosphoglycerate (3PG; Figure 1). Glutamine, the most abundant of all amino acids, is another source of building blocks for macrosyntheses. Glutamine can be metabolized in the TCA cycle in a process called glutaminolysis, providing carbons for amino acids and lipids. Moreover it is important nitrogen source for the cells (Figure 1)[6,7]. In addition to glucose and glutamine, other amino acids can also provide TCA intermediates and lipids can provide acetyl-CoA through mitochondrial FA oxidation (FAO).

METABOLISM OF CANCER CELLS

The most pronounced change in cancer metabolism was observed almost a century ago by Otto Warburg. Normal cells metabolize glucose in the mitochondrial TCA cycle. Only under low oxygen, glucose is converted into lactate. Warburg found that, in contrast, cancer cells metabolize glucose into lactate even in the presence of oxygen, which is referred to as aerobic glycolysis or "the Warburg effect" (Figure 2)[8,9]. This sustained aerobic glycolysis in cancer cells is linked to the activation of oncogenes or loss of tumor suppressors, with PI3K/AKT/mTOR pathway, c-Myc, HIF-1α and AMPK as major players[10-16]. Interestingly, not only cancer cells but also highly proliferating nontransformed activated lymphocytes, thymocytes and embryonic cells metabolize glucose to lactate even in the presence of oxygen[17-21]. This indicates that the Warburg effect is a common feature of proliferation rather than a unique tumor characteristic. Yet, to achieve aerobic glycolysis, tumors upregulate cancer-specific enzyme isoforms, which may provide a therapeutic window for selective anti-tumor therapy. In order to compensate for the lower efficiency of energy generation in glycolysis (2 ATP compared to 36 ATP from mitochondrial respiration for each glucose molecule catabolized), cancer cells upregulate glucose transporters, particularly GLUT1 and GLUT3 to take up more glucose[22]. In fact, this dramatic increase in glucose uptake is exploited clinically to visualize tumors by 2-(18F)-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET). In addition, cancer cells elevate the expression of several glycolytic enzyme isoforms, including HK2, PFKFB3 and M2 isoform of PK (PKM2, Figure 1). While HK1 is found in all mammalian tissues, HK2 is normally restricted to the skeletal muscle and adipocytes. Cancer cells show not only increased expression of HK2 but they also rely on this particular isoform[23-26], indicating an additional role of HK2 in the context of malignancies. The observation that the mitochondria-associated form of HK influences the regulation of apoptosis may reflect one such activity[27]. PFKFB3 is an another example of a regulatory enzyme in glycolysis being upregulated in several cancers[28]. By generating fructose-2,6-bisphosphate (F-2,6-BP), PFKFB3 activates PFK1 to increase the flux through this step of glycolysis. Most isoforms of PFKFBs are bifunctional enzymes with both kinase and phosphatase activities, and can therefore also catalyze the destruction of F-2,6-BP and decrease PFK1 activity[29]. Notably, the PFKFB3

isozyme has low phosphatase activity and therefore activates PFK1 to trigger flux through glycolysis. Another important controller of the glycolytic flux in cancer is PK, an enzyme catalyzing the final irreversible step of glycolysis-conversion of phosphoenolpyruvate (PEP) to pyruvate with concomitant generation of ATP. Whereas differentiated tissues express the M1 isoform of PK (PKM1), all cancers express the M2 isoform (PKM2)[30]. PKM2 promotes aerobic glycolysis, and PKM2-expressing cells show a selective growth advantage *in vivo* relative to PKM1-expressing cells[31]. PKM1 is a constitutively active enzyme, whereas PKM2 enzyme activity is inhibited following its binding to tyrosinephosphorylated proteins downstream of cellular growth signals[32]. Interestingly, PKM2-depleted tumors continue to proliferate only in the absence of PKM1, suggesting that it is the inactivation of both PKMs (or their deficiencies) that underlies tumor growth[33]. The inhibition of PKMs might help to divert glucose-derived metabolites upstream of PK into biosynthetic pathways[34]. In this regard, accumulating G6P can fuel PPP to synthesize nucleotides and NADPH, and DHAP can be used for lipid synthesis and 3PG for amino acids (Figure 1). Paradoxically, PKM2-expressing cells seem to generate more glucose-derived lactate relative to cells expressing PKM1. This process was suggested to be mediated by the upregulation of an alternative pathway that converts PEP to pyruvate without producing ATP, and therefore compensates for the decreased PKM2 activity in proliferating cells[35]. Moreover, LDH isoform A (LDHA), an enzyme responsible for the conversion of pyruvate to lactate, was shown to be upregulated in several tumors (Figure 1)[36]. The conversion of pyruvate to lactate is beneficial for cancer cells as it regenerates NAD+ to accelerate glycolysis and decreases ROS levels, thereby diminishing oxidative stress and promoting tumors' survival[37-39]. Moreover, lactate secreted by one tumor cell can be taken up and oxidized in the TCA cycle of a well-oxygenated neighboring tumor cell[40].

To support aerobic glycolysis, cancer cells stimulate not only glycolytic enzymes, but also actively inhibit glucose-derived pyruvate oxidation in the TCA cycle. They do so by deactivating PDH, an enzyme that catalyzes pyruvate oxidation in the mitochondria and thus keeps it away from lactate production (Figure 1). Indeed, the expression of pyruvate dehydrogenase kinase 1 (PDK1), a negative regulator of PDH, is increased in several cancers[41,42]. Besides increasing lactate production, PDH inhibition diminishes OxPhos and ROS production, resulting in the decrease of oxidative stress. Conversely, reactivation of PDH upon PDK1 inhibition was shown to decrease lactate production and induce oxidative stress and cancer cell death[43,44].

Although oxidative metabolism of glucose is suppressed in cancer cells, the mitochondrial activity is normally conserved. Nonetheless, several genes encoding TCA cycle-related enzymes were found to contain mutations that support malignancy. For example, genomic analyses of tumors have implicated specific mutations of isocitrate dehydrogenases (IDH) isoform 1 and 2 as tumorigenic drivers[45-48]. IDHs are enzymes that normally catalyze the

conversion of isocitrate to αKG with the concomitant production of NAD(P)H (Figure 1). The mutant IDH1/2 enzymes catalyze the normal IDH reaction very inefficiently. Instead, they convert αKG to the rare metabolite 2-hydroxyglutarate (2-HG) while oxidizing NAD(P)H to NAD(P)$^{+}$[49,50]. Growing evidence suggests that 2-HG promotes tumorigenesis, at least in part, by the competitive inhibition of various αKG-dependent dioxygenases that control gene expression epigenetically. They include the TET family of methylcytosine dioxygenases and histone lysine demethylases (KDM), enzymes that regulate the methylation state of DNA and histones, respectively[51-53]. Consistent with this notion, cells with IDH mutations were reported to have an altered methylation pattern and 2-HG-mediated inhibition of histone demethylation blocks the differentiation of untransformed cells[54-56]. Also, loss-of-function mutations in the TCA cycle enzymes succinate dehydrogenase (SDH) or fumarate hydratase (FH) have been linked to tumorigenesis (Figure 1). Succinate or fumarate, which accumulate in mitochondria owing to the inhibition of SDH or FH, overflow to the cytosol, where they inactivate prolyl hydroxylases (PHDs), a subset of the αKG-dependent dioxygenase family[57-59]. Inactivation of PHDs leads to accumulation of the central regulator of oxygen-sensing pathway HIF-1α, which promotes metabolic reprogramming towards tumor-supporting aerobic glycolysis[60,61].

In addition to glycolysis, cancer cells also rely on glutaminolysis to meet their highly energetic demands for proliferation[7]. The products of glutaminolysis are very important in fueling the TCA cycle of tumors. In this way, glutamine provides intermediates for the synthesis of FA and amino acids, essential building blocks of lipids and proteins, respectively. In fact, cancer cells increase glutamine uptake by the upregulation of ASCT2 and SN2, two important glutamine transporters (Figure 1)[62,63]. Moreover, glutaminase 1 (GLS1), a key enzyme of glutaminolysis, is highly expressed in cancer cells and its silencing delays tumor growth (Figure 1)[64-66]. Likewise, cancer cells are highly sensitive to glutamine withdrawal[67].

Highly proliferating cancer cells are also in constant need for nucleotides to replicate DNA. By providing pentose phosphate for nucleotide synthesis, the PPP is important and, in agreement with this, is frequently upregulated in many types of tumors[68]. Accordingly, the activity and levels of G6PDH, a rate-limiting PPP enzyme, are increased in multiple types of cancer (Figure 1)[69,70]. Similarly, the levels of transketolase, another PPP enzyme, were shown to be elevated, too[71]. In addition to building blocks for nucleotides, the PPP promotes tumor survival by producing NADPH, a key tool for both preventing oxidative stress and biosynthesis[68].

Increased lipid turnover is another remarkable feature of cancer metabolism. Most normal cells preferentially use circulating FA for the synthesis of new structural phospholipids. Cancer cells, however, frequently upregulate *de novo* FA synthesis to satisfy high phospholipid needs for the generation of new membranes[72]. Notably, virtually all esterified FAs in tumor models are derived from *de novo* synthesis[73]. High rates of tumor lipogenesis

are reflected by increased activity and expression of several lipogenic enzymes. FASN, a key lipogenic enzyme responsible for the terminal step in FA synthesis, is elevated in several tumors and its inhibition dampens xenograft growth in mice (Figure 1)[74-76]. Similarly, ACLY, an enzyme necessary for exporting citrate from the mitochondria to the cytosol for *de novo* lipogenesis, is indispensable for tumor formation both *in vitro* and *in vivo* (Figure 1)[77,78]. Additionally, ACC is important for tumorigenesis, as its inhibition induces growth arrest and apoptosis of cancer cells (Figure 1)[79,80]. Notably, metabolism of choline, which, next to FAs, is an important precursor of cellular membrane phospholipids, is also altered in cancer cells. Several enzymes of choline metabolism are overexpressed and/or activated and therapeutic response of tumors can be monitored by magnetic resonance spectroscopy (MRS) of the total and phosphocholine levels[81]. In addition to the higher FA synthesis, upregulation of FAO has been linked to tumorigenesis. ATP production by FAO has been shown to prevent anoikis, a type of apoptotic cell death due to loss of attachment to the extracellular matrix[82]. Poly ADP ribose polymerase (PARP)-mediated activation of FAO correlates with poor survival of breast cancer patients[83]. Along these lines, the expression of carnitine palmitoyltransferase-1 isoform C (CPT1), an enzyme stimulating FAO, is higher in cancer cells, leading to increased FAO-derived ATP production and resistance to glucose deprivation (Figure 1)[84]. At the same time, depletion of CPT1C decreases tumor growth *in vivo* and increases sensitivity to metabolic stress. In short, cancer cells display a specific lipogenic phenotype essential for the malignant phenotype and this is therefore, in addition to the anaerobic glycolysis, an important hallmark of cancer.

SENESCENCE AS A POTENT ANTITUMOR MECHANISM

Several mechanisms have evolved to prevent malignant transformation. In response to a tumorigenic insult, cells can undergo various cell death programs such as apoptosis[85] or autophagy[86]. Alternatively, they can stop proliferating and enter a program called senescence. Cellular senescence is a state of irreversible arrest of the cell cycle that involves an activation of tumor suppressor pathways, including $p16^{INK4A}$-Rb and ARF-p53[87-90]. Conversely, expression of cell-cycle activators cyclin A, cyclin B and PCNA is downregulated[91]. Senescence is also accompanied by morphological changes, the induction of senescence-associated β-galactosidase activity (SA-β-Gal)[92], chromatin condensation into senescence-associated heterochromatin foci (SAHF)[93,94] and the activation of a secretory program called senescence-messaging secretome (SMS) or Senescence-Associated Secretory Phenotype (SASP)[95-101].

Senescence was originally identified as a process associated with exhaustion of replicative potential[102]. This type of irreversible cell cycle arrest, termed 'replicative senescence', has been suggested to represent a failsafe mechanism preventing the expansion of aged cells[103]. Later, it was shown that this process involved the erosion of telomeres; and that senescence

can also be induced prematurely (that is, in the presence of normal functional telomeres) by various stress signals, including DNA-damaging insults[104,105] and oncogenic signaling triggered by the activation of an oncogene or the inactivation of a tumor-suppressor gene[87]. Research carried out in more recent years has clearly demonstrated that oncogene-induced senescence (OIS) is a powerful *in vivo* tumor-suppressing mechanism both in model systems and humans. For example, the finding that oncogenic RAS induces OIS *in vitro* was validated *in vivo* using mouse models. Expression of oncogenic *KRAS* from its endogenous promoter in mice results in the development of lung adenomas, as well as premalignant pancreatic intraductal neoplasia, which rarely progress to malignancy [106]. These benign lesions show a low proliferative index and express several senescence markers, including the activation of SA-β-Gal and upregulation of $p15^{INK4B}$ and $p16^{INK4A}$. Another RAS family member, HRAS, also induces senescence *in vivo*. Inducible expression of an oncogenic *HRAS* transgene in the mammary gland induces proliferation when the oncogene is present at low levels, but tumor cell senescence when the oncogene is highly expressed[107]. Moreover, expression of oncogenic *HRAS*, whether expressed from its endogenous promoter or directed to the bladder epithelium, leads to tumor cell senescence[108,109]. In addition, murine papillomas induced upon DMBA/TPA treatment (leading to RAS mutation), express several senescence markers[106,110,111]. Along these lines, senescence features are observed in mouse models with oncogenic BRAF, a proximal RAS downstream kinase. Conditional expression of oncogenic BRAF from its endogenous promoter in melanocytes causes the formation of nevus-like benign lesions[112,113]. These nevi express markers of senescence and remain stable for several months. Consistent with this, oncogenic BRAF expression in lungs initially induces proliferation, but is followed by proliferative arrest associated with characteristics of senescence[114].

In addition to RAS oncogenes and their downstream kinases, also distal effectors of the RAS pathway induce senescence in murine tumors. For example, the expression of an E2F3 transgene in the pituitary gland of mice causes an initial phase of proliferation, but cells successively stop dividing, acquire markers of senescence and fail to form tumors[115]. Also activation of PI3K/AKT/mTOR pathway triggers a senescence response. In fact, *PTEN* was the first example of a tumor suppressor gene whose loss triggers senescence *in vivo*. Conditional *PTEN* deletion in murine prostate cells results in the formation of high-grade prostate intraepithelial neoplasia (PIN), which displays characteristics of senescence. In combination with p53 loss, however, these lesions progress to malignant prostate carcinomas[116]. Similarly, the expression of AKT1 in the prostate results in the development of PIN lesions, which have features of cellular senescence[117]. Likewise, mice overexpressing RHEB, which connects AKT to mTOR, also triggers PIN lesions that are positive for senescence markers[118].

Oncogene inactivation may also induce cellular senescence: enforced loss of Myc in Myc-initiated hepatocellular carcinomas, lymphomas or osteosarcomas, causes tumor regression that is associated with the induction of senescence[119].

Another example of cellular senescence *in vivo* comes from kidneys from von Hippel-Lindau (VHL) knockout mice, which display an increase in SA-β-Gal activity and levels of $p27^{Kip1}$ and DcR2, a TNF family decoy receptor associated with senescence[120]. Also genetic loss of one allele of Rb drives formation of thyroid adenomas that have several senescence markers[121]. Cell senescence has also been reported in humans. The (benign) melanocytic nevus was the first human lesion for which the evidence was shown in favor of the idea that OIS prevents malignant progression[122,123]. In spite of the presence of oncogenic BRAF (or, in some cases, NRAS), nevi are commonly cell cycle arrested and exhibit evidence of senescence markers, such as SA-β-Gal and $p16^{INK4A}$. Lesions suffering from loss of the tumor suppressor neurofibromin 1 (NF1) are another example of cellular senescence in humans[124]. As NF1 is a negative regulator of RAS activity, loss of NF1 results in hyperactivated RAS signaling and the development of neoplastic lesions, known as neurofibromas, which show markers of senescence. Finally, premalignant human colon adenomas and PIN lesions also show features of senescence including increased SA-β-Gal activity and induction of SMS components[95,96].
As described above, numerous studies have demonstrated that senescence is associated with pre-malignant stages of neoplastic transformation and has a crucial role in preventing tumor development. Hence, understanding the mechanisms controlling the senescence program is of vital importance as it may guide us to novel therapeutic targets in cancer. However, despite enormous efforts, we have only begun to uncover some of the underlying principles, and many questions remain to be answered. Among processes that remain largely unexplored in senescence, the ones controlling cellular metabolism are at the forefront.

METABOLISM OF SENESCENT CELLS

Proliferating and senescent cells are expected to have very different metabolic requirements. Considering that proliferating cells must duplicate their cellular biomass in order to divide, much of their metabolic energy is devoted to synthesis of DNA, proteins and lipids. Senescent cells appear to be relieved of this vast metabolic demand because they are not dividing. However, induction of senescence is accompanied by a multitude of specific morphologic and physiologic changes that involve energy-consuming processes, too. Accordingly, strongly induced production of, for example, senescence-associated cytokines must require shuffling of metabolic resources towards protein synthesis. Also, vesicular transport and secretion, both active in senescent cells, demand sufficient supply of the energy. Hence, while freed from the biosynthetic needs accompanied with creating daughter cells, senescent cells still must adopt their cellular metabolism to support energetic and anabolic requirements. Markedly, regardless of shared belief that senescent cells are metabolically active, relatively few studies have meticulously investigated senescence-associated changes of metabolism (Table 1).

Table 1. Metabolic alterations in senescence

Type of senescence	Metabolic alteration	Reference
replicative	strong decrease in glycolysis and production of glucose-derived lactate	125
RAS-induced	ectopic expression of glycolytic enzymes, PGM or GPI, increases glycolytic flux, decreases oxidative damage and abrogates senescence; downregulation of glycolysis upon depletion of PGM or GPI induces senescence	126
	despite lower rate of *de novo* synthesis of FA, OIS cells show increased steady-state levels of free FA due to the higher FA oxidation	139
	suppressed nucleotides metabolism due to the downregulation of RRM2; ectopic expression of RRM2 restores nucleotide metabolism and abrogates senescence	135
p53-dependent	p53 induces the expression of TIGAR, an enzyme diminishing glycolytic activator F-2,6-BP; this inhibits glycolysis leading to cell cycle arrest	127
	p53 represses the expression of TCA cycle-associated ME1 and ME2; depletion of ME1 and ME2 reciprocally activates p53 leading to a strong induction of senescence	132
therapy-induced	chemotherapy-induced senescence associates with enhanced glucose utilization in the TCA cycle	129
	growth arrest induced by inhibition of melanoma-driver BRAF is accompanied by several senescence features and increased oxidative metabolism	130,131
BRAF-induced	increased TCA cycle activity due to the activation of mitochondrial gatekeeper PDH; PDH is activated upon downregulation of its inhibitory kinase PDK1 and simultaneous upregulation of PDH-activating phosphatase PDP2; normalization of the levels of these enzymes inactivates PDH resulting in abrogation of OIS	133
replicative, DNA damage-induced and oncogene-induced senescence	increased ratio of GPC to PC, two important components of choline metabolism	140

GPC - glycerophosphocholine; GPI - glucosephosphate isomerase; FA - fatty acids; F-2,6-BP - fructose2,6-bisphosphate; ME - malic enzyme; OIS - oncogene-induced senescence; PC - phosphocholine; PDH - pyruvate dehydrogenase; PDK1 - pyruvate dehydrogenase kinase 1; PDP2 - pyruvate dehydrogenase phosphatase 2; PGM - phosphoglycerate mutase; RRM2- ribonucleotide reductase subunit M2; TCA cycle - tricarboxylic acid

It has been reported that senescent cells adapt their metabolic profiles, which seem to oppose the ones seen in the cancer cells. In this regard, senescence manifests its antitumor function also at the level of metabolic (de)regulation. While tumor cells upregulate aerobic

glycolysis, replicative senescence is accompanied by strong decreases in glycolytic flux and production of glucose-derived lactate[125]. Downregulation of glycolysis induces metabolic imbalance, which is associated with decreased levels of ribonucleotide triphosphates, thereby leading to senescence-associated cell cycle arrest. Consistent with these observations, modulation of glycolytic enzymes controls the senescence response[126]. A function-based screen for immortalizing genes identified the glycolytic enzyme PGM to be crucial for abrogation of RAS-induced senescence (Figure 1). Analysis of the impact of other glycolytic enzymes on cell cycle arrest showed that glucosephosphate isomerase (GPI) can drive senescence escape (Figure 1)[126]. Along these lines, ectopic expression of PGM or GPI increases glycolytic flux, decreases the oxidative damage and extends the life span of primary fibroblasts. Conversely, downregulation of glycolysis upon depletion of PGM or GPI induces premature senescence[126]. Similarly, TIGAR, an enzyme diminishing glycolytic activator F-2,6-BP and thereby glycolysis, was reported to induce cell cycle arrest, a key characteristic of senescent cells (Figure 1)[127,128].

Growing evidence indicates that senescence provokes an anti-Warburg effect not only by downregulation of glycolysis but also by altering TCA cycle activity. In fact, therapy-induced senescence (TIS) has been associated with enhanced glucose utilization in the TCA cycle[129]. Along these lines, cell cycle arrest upon inhibition of the common melanoma driver oncogene BRAF is accompanied by the induction of several senescence features[130] and an increase in oxidative metabolism[131]. Moreover, p53-dependent senescence has been linked to the regulation of TCA-associated malic enzymes (MEs, Figure 1)[132]. While p53 accumulation represses the expression of ME1 and ME2, depletion of ME1 and ME2 reciprocally activates p53, resulting in a strong induction of senescence. In addition, we have demonstrated that OIS cells show an increased TCA cycle activity due to the activation of PDH, an enzyme linking glycolysis to oxidative phosphorylation[133]. In OIS, PDH is activated upon downregulation of its inhibitory kinase PDK1 and simultaneous upregulation of PDH-activating phosphatase PDP2 (Figure 1). Normalization of the levels of these enzymes inactivates PDH, resulting in abrogation of OIS. Likewise, the analysis of changes in protein expression in OIS revealed upregulation of proteins involved in oxidative phosphorylation and downregulation of proteins involved in glycolysis, further supporting an important role of oxidative metabolism in senescence[134].

Regulation of nucleotides-producing PPP has also been reported to play a role in the senescence response. Ribonucleotide reductase subunit M2 (RRM2), a PPP enzyme crucial for nucleotide synthesis, is downregulated in senescence, leading to a sharp decrease in the number of nucleotides available for DNA synthesis required for cell cycle progression (Figure 1)[135]. The suppression of nucleotide metabolism represents a critical element underlying the establishment and maintenance of OIS. Accordingly, either addition of exogenous nucleosides or restoration of RRM2 abrogates RAS-induced senescence. This indicates that,

in contrast to high PPP activity in cancer cells, the PPP must be shut down for senescence induction, as interference with this regulation abrogates the senescence program. Similarly, depletion of 6-phosphogluconate dehydrogenase (6PGD), the third enzyme in the PPP, inhibits proliferation of lung cancer cells *in vitro* and in mice due to the induction of senescence as evidenced by the upregulation of the senescence markers SA-β-Gal, p53, and $p21^{Cip1}$ (Figure 1)[136].

As described above, cancer cells strongly depend on glutaminolysis for growth and proliferation. In contrast, senescence has been linked to the inhibition of glutamine metabolism. Endothelial cells rely heavily on glutaminolysis. The removal of this energy source by pharmacological inhibition of GLS, a key enzyme in glutaminolysis, drastically decreases cellular ATP levels, leading to the induction of cell cycle arrest, enhanced SA-β-Gal activity and induction of $p16^{INK4A}$ and $p21^{Cip1}$ protein levels in these cells[137]. Hence, downregulation of glutamine utilization likely represents a tumor-suppressive response.

Lastly, several studies have recently linked senescence to changes in lipid metabolism. Replicative senescence is accompanied by reduced biosynthesis of FA and formation of phospholipids[138]. Also, OIS cells have been demonstrated to have a lower rate of *de novo* synthesis of FA[139]. Despite that, OIS cells show increased steady-state levels of free FA due to the higher FAO. Inhibiting the FAO, however, did not prevent senescence-associated cell cycle arrest, arguing against causal role of FA in senescence program[139]. In addition, senescent cells were recently reported to have altered choline metabolism. Analysis of metabolic changes in three types of senescence: replicative, DNA-damaged induced and OIS, showed that the ratio of glycerophosphocholine (GPC) to phosphocholine (PC) is increased independently of the senescent type[140]. Notably, this is diametrically opposite to the change in choline metabolism in tumor cells. During malignant transformation cells show “GPC to PC” switch implying an increase of PC compared to GPC[141-143]. Conversely, chemotherapy-mediated induction of cell cycle arrest and apoptosis in cancer cells is accompanied by the increase in GPC levels[144]. This implicates that suppression of choline metabolism in senescence serves as an antitumor mechanism, too.

CONCLUDING REMARKS

Research performed in recent years has made it clear that cancer cells rewire their metabolism to support growth and proliferation. This remodeling goes beyond upregulation of aerobic glycolysis but involves all major cellular metabolic pathways. The observation that several of the oncogenic and tumor suppressor pathways regulating metabolism in tumor cells are linked also to senescence, suggested that the latter program must, somehow, be mechanistically connected to metabolic (de)regulation. Indeed, a number of studies on metabolic changes in senescence have revealed that metabolic pathways altered in cancer cells are also important for the execution of the senescence program. In fact,

the metabolism in senescent cells is rewired in a way that counteracts changes required for malignant transformation. Hence, the antitumor function of senescence is manifested also at the level of metabolic regulation. Nevertheless, we have only begun to undercover the mechanism that controls metabolism in the senescence setting. Further research on metabolic alteration in senescence will be necessary to better understand how cancer cells disrupt the normal limitations of metabolism control. This will hopefully reveal new therapeutic metabolic cancer targets.

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